LARVAL TREMATODES FROM AUSTRALIAN FRESHWATER MOLLUSCS
PART XIII

By T. Harvey Johnston and L. Madeline Angel*

[Read 12 May 1949]

Cercaria beckwithae n. sp.

On 27 October 1948, 6 of 49 *Planorbis isingi* collected from a small artificial rock pool in the garden of Mr. G. Jaensch, Tailem Bend, were found to be giving off stylet cercariae of a type not previously encountered by us. This pool is fed with water pumped from the neighbouring swamps, being filled up approximately once per fortnight during the summer months. It is several years since snails were introduced into the pool by Mr. Jaensch, and as the life span of *Planorbis isingi* appears to be under two years, it follows that infection of the snails must have occurred in the pond itself.

From 27 October 1948 to 24 January 1949 *C. beckwithae* has been identified from 16 of 403 snails—approximately a 4% infection. It was not present in any of 431 *Planorbis* collected from the same pond at the end of February 1949. It is of interest that the only other kind of gastropod present in the pond, *Amerianna* sp., is evidently not a suitable host for this cercaria since none of these snails was found infected with it. This cercaria has not been found in *Planorbis* collected from the swamps along the lower River Murray. As will be discussed later, we expect to find that the adult is a frog lung fluke and experiments to ascertain the life history will be continued. That the infection was not an isolated case is indicated by the following facts:—(1) that of 69 *Planorbis* collected on 30 November 1948 and apparently negative when tested then, and again a week later, one was found to be giving *C. beckwithae* when next tested on 23 December; (2) that the one *Planorbis* which was positive from among 180 collected on 24 January 1949, was not quite half-grown. This latter must certainly have been extremely small when the original infection found by us had taken place, and would have been unlikely to survive an infection at that stage.

The Cercaria

The cercaria is small, an average of 20 specimens fixed in boiling 10% formalin in the standard manner being 165μ by 100μ wide. The range (105μ by 105μ to 240μ by 86μ) is considerable, because some cercariae are fixed in greatly extended position, while others are completely contracted. The tail averaged 162μ by 32μ; range 112μ by 30μ to 202μ by 37μ. The oral sucker averaged 47μ long by 49μ wide, while the acetabulum was 29μ by 32μ, giving an approximate sucker ratio of 5:3. The acetabulum lies in the posterior half of the body. The stylet is rather delicate in appearance; length 32μ; width at base 5·3μ; width at rim, formed approximately at the end of the anterior third, 4·9μ.(1) The tail is inserted on the ventral surface of the body and is provided with a transparent finfold dorso-ventrally placed and extending a very little distance around the tip on the dorsal side, but for about a third of the length of the tail on the ventral

* University of Adelaide.

(1) In this regard we may state that observations on xiphidiocercariae examined in this department corroborate the observation of Brooks (1943) that he has been "impressed with the uniformity of the dimensions of the stylets of various species" and believes that "greater use can be made both of the shape and length of the stylet in describing and identifying cercariae of this group." Further, the size of the stylet is not altered by prolonged immersion in formalin, as we have verified with at least two kinds of xiphidiocercariae.

(fig. 5). When the tail is at rest, the flange is fluted. Under coverslip pressure, the tail tends to keel over to give the appearance of a laterally placed flange. The tail stains blue with nile blue sulphate but is uncoloured with neutral red.

The body is fairly clear; there are no coloured refractile granules as are seen in many xiphidiocercariae. The surface is beset with minute spines, though these are so small as to be indicated only under oil immersion magnification and under favourable conditions of intra-vitam staining. Ordinary methods recommended to show spines, *e.g.*, the use of picric acid and menthol, were ineffective. There was no indication of the fine protoplasmic hairs described by some writers for related cercariae. Caudal pockets are not present.

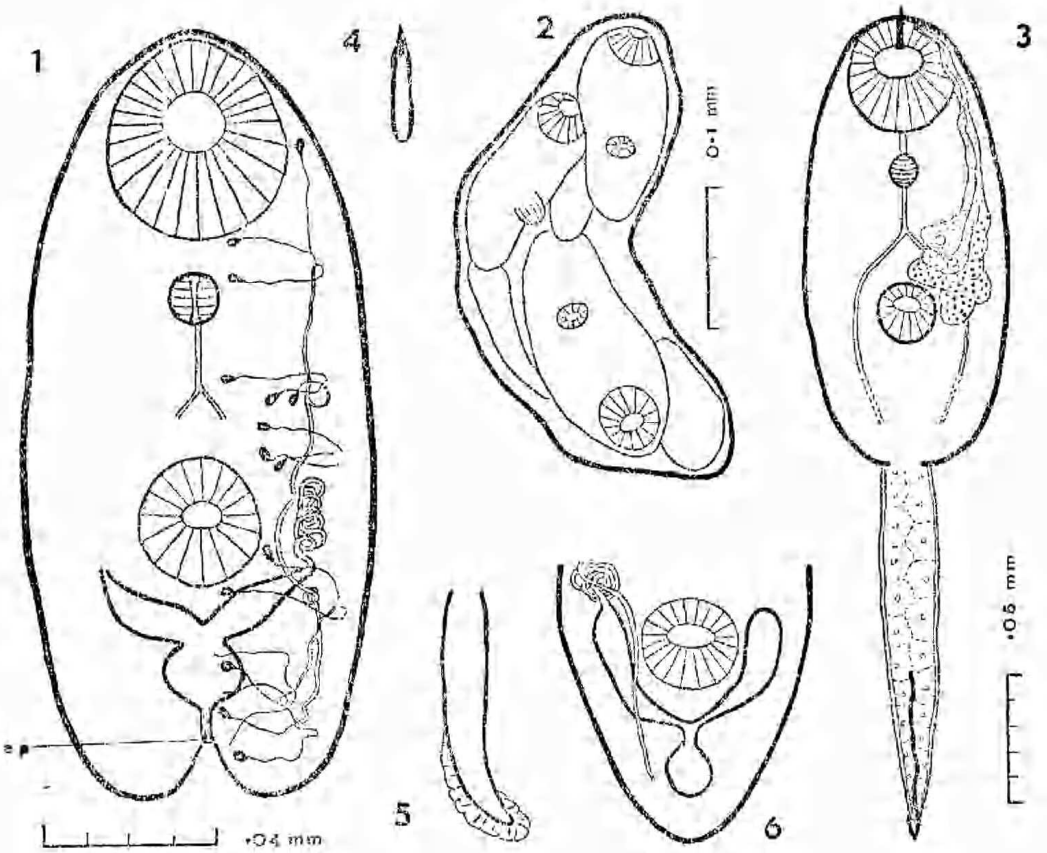

Fig. 1: body of cercaria, outlines from camera lucida drawing—details of excretory system from living specimens. Fig. 2: sporocyst. Fig. 3: cercaria, gland cells and alimentary system. Figs. 4, 5, 6: sketches. 4, stylet. 5, tail in dorso-ventral view. 6, excretory cornua in more extended position.

Reference to lettering: ep = excretory pore.

On either side of the body there is a group of gland cells extending from the bifurcation of the oesophagus almost to the level of the posterior border of the acetabulum. It is impossible to determine the number accurately, but there are from 3 to 5 (perhaps more) pairs. Specimens stained with nile blue sulphate following neutral red show two pairs anteriorly and medially which are finely granular but uncoloured, while the remaining gland cells take on a dirty purple

colour; these and their ducts, however, tend to contract into an indeterminate mass. In unstained specimens the nuclei of the glands appear clear and are slightly tinged with pink. Throughout the body there are a number of other cells, which are presumably cystogenous, and under extreme coverslip pressure when the nuclei become evident it is not possible to distinguish such nuclei from those of the gland cells in the same region.

There is a short pre-pharynx, a quite circular pharynx and a very narrow oesophagus which bifurcates some distance anteriorly to the acetabulum. The angle of bifurcation is characteristically acute (figs. 1, 3); the crura are very narrow, and in living specimens are not seen beyond the level of the posterior border of the acetabulum, and rarely as far as its anterior border. Staining rendered them slightly more obvious, and in a few of the best preparations they could be seen to extend almost to the end of the body, ending level with the insertion of the tail. Krull (1933) when describing the cercaria of *Haematoloechus complexus*, indicated very narrow intestinal crura and noted that they were very difficult to see, even in the most favourable specimens.

The excretory bladder is Y-shaped; the arms of the Y terminating normally below the level of the anterior border of the acetabulum, but in some specimens (notably in those which had been swimming in a solution of basic fuchsin in normal saline) the arms were well above this region. There is, of course, a considerable margin of difference between the levels reached in the expanded and contracted positions of the bladder. The main excretory tubes are attached at the anterior tips of the arms. The flame cell formula is apparently $2\,[\,(3+3+3)+(3+3+3)\,]$. This is extremely difficult to determine, and for a long time we thought that there were only two groups each of three flame cells, attached to the anterior collecting tubule. When the third group from the anterior end was seen its point of origin from the collecting tubules could not be determined, and we are assuming that it is attached to the anterior tubule, as seems most likely. Again, the point of bifurcation of the anterior and posterior collecting tubules has not been seen definitely, though we feel satisfied that it is on a level just behind the anterior border of the acetabulum in a position where the convolutions of the main excretory tubule rendered any closer elucidation impossible. In the posterior groups not all the flame cells have been seen; the last two groups however are clearly indicated by the capillaries. In the first of the posterior groups only two of the elements have been seen, but we have no doubt that a third is present. The excretory pore opens at the base of the tail by a crescentic slit on the ventral surface. There is no caudal excretory tube as shown by Sewell for several xiphidiocercariae. In stained, fixed specimens the genital rudiment shows as an irregular undifferentiated mass dorsal to the acetabulum, and of about the same size.

Experimental Infections

We have not been able to obtain the cyst stage, though a number of different animals have been used for experimental infections. Negative results were obtained with *Daphnia* sp.; Dytiscid beetle larvae; dragon-fly larvae, *Aeschna brevistyla* and *Austrolestes analis;* the yabbie, *Cherax destructor;* mosquito larvae; leeches (*Glossiphonia* spp.); the molluscs, *Ameriana* spp. and the host species, *Planorbis isingi;* as well as with tadpoles and the fish, *Gambusia affinis.*

Cercaria of all frog lung flukes of which the life-history is known encyst in larval insects, the majority in dragon-fly larvae. In some species there is a considerable degree of specificity for the second intermediate host. Krull (1931) found that cercariae of *Haematoloechus medioplexus* and *H. parviplexus* encysted in two species of *Sympetrum* but did not infect closely related dragon-flies. On

the other hand, he (1933) thought it probable that many species of dragon-flies could serve as hosts for *H. complexus*. Ingles (1933) suggested that the presence of the infection of *Ostiolum oxyorchis* in frogs collected from ponds and its absence from frogs of the same species collected from small streams was due to the habits of the intermediate host since most of the natural infections of *O. oxyorchis* occurred in the pond-inhabiting dragon-fly, *Sympetrum illotum*. Such a specificity may well explain the fact that *C. beckwithae* has been found only in a pond and not in the swamps, and also our failure to obtain its metacercaria in the only two species of dragon-fly larvae which were available to us for experiment, and which had been obtained from the swamps. Krull (1932) reported that the metacercaria of *Pneumobites longiplexus* whose adult stage occurs in *Rana* sp., was found in cysts or free in the body cavity of damsel flies, *Lestes* sp. We have not found metacercariae in numerous dragon-fly larvae (*Aeschna brevistyla*) collected from swamps along the Lower Murray.

The Sporocyst

The sporocysts are inconspicuous, and cannot be discerned as finite bodies when the snail is dissected after death. Numbers of cercariae are found in the liver; these apparently migrate from the sporocyst soon after the death of the host, leaving the sporocyst as an empty sac. Staining of some of the dissected liver material gave one good preparation of a sporocyst, a small body containing (and more or less filled by) three or four cercariae (fig. 2). If we had had sufficient material to sacrifice a living snail, the sporocysts would probably have been more obvious.

Affinities

C. beckwithae belongs to the Cercariae Ornatae, a group defined by Lühe (1909) as "distome cercariae with a stylet, in which the slender tail is furnished with a fin fold." Since 1914, when Cort described *C. hemilophura* and included it provisionally in the "Ornatae," workers have stressed the fact that the group is probably an unnatural one.

Sewell (1922) created the "Prima" subgroup, and Faust (1924, table II) in his "synoptic flame-cell formulary for digenetic trematodes" placed *C. hemilophura* Cort 1914 and *C. trifurcata* together in the "Hemilophura group," as having a flame cell formula of $2[\ (3+3)+(3+3+3)\ ]$. It may be noted that Faust (1924) included *Cercaria prima* with *C. indica LIII* Sewell in the "Daswan" group, and thus denied the importance of the fin fold in the classification of cercariae, since *C. indica LIII* has not this feature.

In 1929 McCoy, who did further work on the excretory system of *C. hemilophura* and ascertained the formula to be $2\ [\ (3+3+3)+(3+3+3)\ ]$, found it necessary to remove this cercaria from the group, though he did not create one to contain it.

In 1936, E. L. Miller divided the Cercariae Ornatae into four subgroups, using the flame cell formula as the differentiating feature:—

I. Sewell's Prima group, with an excretory formula $2 \times 6 \times 1$ (*i.e.*, $2\ [\ (3)+(3)\ ]$).

II. Hemilophura (sic) group, containing only *C. trifurcata* Faust 1919; formula $2\ [\ (3+3)+(3+3+3)\ ]$.

III. A third subgroup (formula $2\ [\ (3+3+3)+(3+3+3)\ ]$) containing *C. hemilophura* Cort 1914 and *C. mesotyphla* E. L. Miller 1935. To this can now be added *Cercaria merchanti* Rankin 1939 (the larva of *Haematoloechus* sp.), *C. herberi* McMullen 1938 (quoted in Zool. Rec. 1938 Vermes, p. 94, as *C. herberi*) and *C. beckwithae*.